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PRIMER NOTE

ISOLATION, VIA 454 SEQUENCING, AND CHARACTERIZATION OF MICROSATELLITES FOR *VACHELLIA FARNESIANA* (FABACEAE: MIMOSOIDEAE)[1]

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- *Premise of the study:* We isolated 15 polymorphic microsatellite markers from *Vachellia farnesiana* for use in population genetic studies to determine the native range of the species.
- *Methods and Results:* Initially, 454 shotgun sequencing was used to identify and design primers for 68 microsatellite loci. Of these, we trialed 47 loci in the target species, and 42 (89%) amplified a product of expected size. Fifteen of the 47 loci were screened for variation in 21 individuals from the native range of *V. farnesiana* in southern Mexico and 20 from northwestern Australia. Fourteen loci were polymorphic, with observed heterozygosity ranging from 0.026 to 1.00 (mean = 0.515) and two to 12 alleles per locus (average = 5.2). Cross-amplification was successful in four to 11 loci in three other *Vachellia* species.
- *Conclusions:* The new microsatellite loci will be useful in understanding genetic variation and investigating the role of human-mediated dispersal in the current distribution of *V. farnesiana*.

Key words: 454 GS-FLX; *Acacia farnesiana*; cross-species transferability; microsatellites; shotgun sequencing; *Vachellia farnesiana*.

Mimosa bush, *Vachellia farnesiana* (L.) Wight & Arn. (synonym *Acacia farnesiana* (L.) Willd.), is a woody mimosoid legume with a pantropical distribution. It has several common names in its native range, including mimosa bush, sweet acacia, cassie, and huizache. *Acacia* Mill., if treated in the broad sense (sensu lato [s.l.]), is a large polyphyletic genus, with at least five lineages that may be recognized as genera: *Acacia* sensu stricto (s.s.), *Acaciella* Britton & Rose, *Mariosousa* Seigler & Ebinger, *Senegalia* Raf., and *Vachellia* Wight & Arn. (Maslin, 2008; Bouchenak-Khelladi et al., 2010). The genus *Vachellia* is composed of a predominantly African clade and a predominantly American clade (Bouchenak-Khelladi et al., 2010). *Vachellia farnesiana* is part of the American clade, but has a distribution that extends well beyond the Americas, and it is considered invasive in some countries. Its arrival date in Australia, and hence its status as native or alien, remains unknown, but *V. farnesiana* may have arrived prior to European colonization (Bean, 2007). The Spanish and Portuguese introduced the species to Europe in the 17th century. At this time, the two countries had a strong colonial presence around the Indian Ocean, through which further dispersal of the plant was possible. However, natural ocean currents and pre-European indigenous traders may have played a role in earlier dispersals. Genetic data from *V. farnesiana* may be useful in determining the dispersal pathways of this plant to populations outside of the Americas. Microsatellite markers have been developed previously for the invasive *V. nilotica* (L.) P. J. H. Hurter & Mabb. (Wardill et al., 2004). However, only a total of five loci were developed, and it is unknown how many of these will cross amplify in *V. farnesiana*. It was, therefore, necessary to develop new markers for *V. farnesiana* to facilitate our investigations of population genetics and plant dispersal out of the native range.

METHODS AND RESULTS

Genomic DNA (5 µg) was isolated from one individual of *V. farnesiana* from silica gel–dried leaves with the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Valencia, California, USA) as per the manufacturer's protocol. The DNA was sent to the Australian Genomic Research Facility (AGRF) in Brisbane, Australia, for shotgun sequencing on a Titanium GS-FLX (454 Life Sciences, a Roche Company, Branford, Connecticut, USA) following Gardner et al. (2011). The sample occupied 12.5% of a plate and produced 59,289 individual sequences, with an average fragment size of 307 bp; 1.9% of the sequences contained microsatellites. The raw data from shotgun sequencing were deposited in the Dryad Digital Repository (doi:10.5061/dryad.jd183; Meglécz et al., 2012). We used the program QDD version 1.3 (Meglécz et al., 2010) to screen the raw

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sequences with eight or more di-, tri-, tetra-, or pentabase repeats. Redundant sequences were removed and primers were designed with a specified PCR product length of 80–480 bp using Primer3 (Rozen and Skaletsky, 2000) in QDD; default settings were maintained for all parameters except product length. The software identified and designed primers for a total of 68 loci, of which 47 contained simple repeats and 21 contained tandem repeats (Table 1).

We followed the procedure outlined in Gardner et al. (2011) for further development of the 47 loci containing simple repeats. The 47 loci were trialed for amplification using seven *V. farnesiana* individuals, each from a different population (Appendix 1), and 10-µL reactions containing 1× buffer, 0.5 U HotStar-Taq DNA polymerase (QIAGEN), 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, 250 nM each forward and reverse locus-specific primer, and 10–50 ng genomic DNA. The following PCR conditions were used: 95°C for 15 min; followed by 28 cycles at 95°C for 30 s, 58°C for 90 s, and 72°C for 30 s; and a final elongation step at 60°C for 30 min. PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide. Twenty-eight loci amplified a product of the expected size for all seven samples, with no unexpected secondary bands. These 28 loci were tested for polymorphism using forward primers tagged with 454A sequence tags and 454A sequencing tags labeled with either 6-FAM, NED, HEX, or PET (Applied Biosystems, Foster City, California, USA) following the method of James et al. (2011) and were run by Macrogen (Seoul, Korea) on a 3730xl DNA sequencer (Applied Biosystems) with a GeneScan 500 LIZ Size Standard (Applied Biosystems). Of the 28 loci tested, 26 loci (93%) were polymorphic, one (3.6%) was monomorphic, and one (3.6%) did not amplify for all samples under these conditions. Of the 26 polymorphic loci, 11 (42%) produced alleles that were affected by stuttering or amplified weakly and were removed from further consideration. The remaining 15 (54%) polymorphic loci (Table 1) were screened for variation in 20 recently collected individuals from a single population from southern Mexico, one herbarium specimen also from southern Mexico, and 20 recently collected individuals from northwestern Australia (Table 2), with DNA isolation, PCR, and fragment length analysis as described above. For each locus, we calculated the number and range of alleles, observed (H_o) and expected heterozygosity (H_e), and deviation from Hardy–Weinberg equilibrium (HWE) using GenAlEx (Peakall and Smouse, 2006). *P* values from HWE tests were adjusted for multiple tests of significance using the sequential Bonferroni method (Holm, 1979). The number of alleles per locus ranged from one to 12 across these 41 individuals, and H_e ranged from 0 to 0.84. Within the Mexican samples, seven polymorphic loci were in HWE, five significantly deviated from HWE, and three were monomorphic. Within the Australian samples, nine polymorphic loci were in HWE, five significantly deviated from HWE, and one was monomorphic (Table 2). We used MICRO-CHECKER 2.2.3 (van Oosterhout et al., 2004) to check each locus for further evidence of null alleles, scoring error due to stuttering, and large allele dropout. Four loci (Af03, Af47, Af32, Af26) showed significant null allele frequencies at the target site, or evidence of scoring error due to stuttering. None of the loci showed evidence of large allele dropout. We checked all pairs of loci for linkage disequilibrium in GENEPOP and none were significant after sequential Bonferroni adjustment.

Primers for the 15 selected loci were also tested for amplification and cross-species transferability in 12 individuals of *V. nilotica* (8 recently collected and 4 herbarium specimens), two herbarium specimens of *V. aroma* (Gillies ex Hook. & Arn.) Seigler & Ebinger, and one each of the Australian species *V. ditricha* (Pedley) Kodela and *V. suberosa* (A. Cunn. ex Benth.) Kodela (Appendix 2). Isolation of DNA, PCR, and fragment analysis were as described above. Thirteen of the 15 loci amplified successfully in the majority of individuals of *V. nilotica*, and eight of these were polymorphic for the small number of individuals examined. Amplification success was lower for the remaining species (5–12 of 15 loci), possibly due to the use of DNA isolated from herbarium specimens.

CONCLUSIONS

These markers will be used to document the genetic diversity of *V. farnesiana* and to investigate the dispersal pathways leading to its current pantropical distribution. Given the successful

Table 1. Characterization of 15 polymorphic microsatellite loci of *Vachellia farnesiana.*

Locus[a]	Primer sequences (5′–3′)	GenBank accession no.	Repeat motif	Allele size (bp)	T_a (°C)
Af18^V	F: GCCACAACTAAAGTCATATCACCA R: CCTTCTTACGCTCCATGATTC	KF030919	$(TA)_9$	108	58
Af24^P	F: CATGGCCTATTTCCACCACT R: TTGGTGCAATTGATAGCGTT	KF030921	$(AT)_9$	94	58
Af05^P	F: TTGGACATTCCAATTGAGATTATTA R: AGCAGGAACTTGCTTAGATGC	KF030916	$(TG)_8$	118	58
Af38^F	F: GATTGCTATGTCATCTCCCTCC R: GTGCGAGATCTATCGACGAC	KF030926	$(GT)_{10}$	98	58
Af19^F	F: ACTTCGAGATGAACCTCCCA R: CGAGACCCAAATCAGTCGAT	KF030920	$(AT)_{11}$	106	58
Af32^N	F: CAGTTCAAACTATCATCTCTATTCACA R: GTGATATGTTTACGGTGCCGA	KF030925	$(AT)_8$	90	58
Af25^N	F: GATGGCGGCAACACAGTAT R: AAGTGAACAATATTGAAGCGCA	KF030922	$(CT)_{10}$	109	58
Af03^N	F: TTAATGCAATTGGGAATCACTT R: GACACTCCCACCTGTATCGG	KF030915	$(GA)_{15}$	150	58
Af10^F	F: GAAGTTATTCTTAATTGCTACCATTCC R: TTGACCAACTCTACTCTTAATTGATTG	KF030917	$(AC)_{12}$	91	58
Af26^F	F: CAGCTCGATAGCTAAACAAGGA R: GGTGTTTGGATGGAAGTTCG	KF030923	$(CA)_{10}$	108	58
Af47^F	F: CCTGAGACAGTTGTGTTTGATTG R: ATCATGCCTTGTCAGCATCC	KF030929	$(AC)_{11}$	121	58
Af14^N	F: ATTACACCACTCGGTCGGTC R: CCCATCTTCTCCAGCATCAT	KF030918	$(AAG)_5$	90	58
Af29^N	F: GGAATCCAATGTATTTGGCG R: AGGTTCACAAGGCAACCTGT	KF030924	$(AT)_8$	109	58
Af42^N	F: AAACTCAATAACTTGCTTAACTGAAA R: CCAATTTGCTTGCTTGACTTG	KF030927	$(TC)_5$	120	58
Af46^N	F: TGAAGAATAATAGCTAGCGGCTG R: TGAGAAGGCCCAATGAAATC	KF030928	$(AG)_9$	91	58

Note: T_a = annealing temperature.

[a] Superscripts F, N, V, and P indicate loci were 5′ labeled with the dyes 6-FAM, NED, VIC, and PET, respectively.

TABLE 2. Genetic properties of 15 microsatellite loci of *Vachellia farnesiana*.[a]

	Mexico						Australia						Total				Amplification of other *Vachellia* species			
Locus	*N*	Allele size range (bp)[b]	*A*	H_o	H_e	HWE *P*	*N*	Allele size range (bp)[b]	*A*	H_o	H_e	HWE *P*	*N*	Allele size range (bp)[b]	*A*	H_o	H_e	*V. nilotica* (*N* = 12)	*V. aroma* (*N* = 2)	*V. ditricha* (*N* = 1)	*V. suberosa* (*N* = 1)
Af18	21	111–127	3	0.95	0.57	0.001*	20	111–129	3	1.00	0.59	0.000*	41	111–129	4	0.98	0.64	P	+	+	+
Af24	21	113–121	5	0.95	0.74	0.031	19	113–121	5	0.79	0.65	0.647	40	113–121	5	0.88	0.75	P	–	–	–
Af05	21	141–155	5	0.19	0.17	1.000	20	141–143	2	0.00	0.10	0.000*	41	141–155	5	0.10	0.14	M	+	–	–
Af38	21	113–117	3	0.10	0.19	0.997	20	113–115	2	0.25	0.22	0.523	41	113–117	3	0.17	0.16	M	+	+	+
Af19	20	122–146	6	1.00	0.68	0.032	16	121–144	8	0.94	0.84	0.489	36	121–146	9	0.97	0.81	M	+	+	+
Af32	20	102–106	2	0.00	0.09	0.000*	18	106–114	5	0.17	0.72	0.000*	38	102–114	6	0.08	0.68	–	–	–	–
Af25	21	124–132	4	0.38	0.46	0.142	18	124–130	4	0.11	0.21	0.006	39	124–132	5	0.26	0.35	P	+	–	–
Af03	21	149–169	4	0.28	0.63	0.000*	18	149–167	6	0.89	0.73	0.0233	9	149–169	7	0.56	0.80	M	–	–	–
Af10	21	93–105	4	0.71	0.68	0.001	20	103–107	3	0.90	0.52	0.004	41	93–107	5	0.81	0.70	–	+	–	–
Af26	21	125	1	0.00	0.00	ND	20	125–129	3	0.10	0.36	0.002	41	125–129	3	0.05	0.20	P	+	–	–
Af47	21	139–149	4	0.71	0.57	0.000*	20	137–147	3	0.10	0.34	0.000*	41	137–149	5	0.42	0.49	P	+	–	–
Af14	20	108–111	2	1.00	0.50	0.000*	20	108–111	2	1.00	0.50	0.000*	40	108–111	2	1.00	0.50	M	+	+	–
Af29	21	124–138	6	0.91	0.77	0.434	20	124–152	9	1.95	0.82	0.003	41	124–152	12	0.93	0.84	P	+	+	–
Af42	20	139	1	0.00	0.00	ND	20	139	1	0.00	0.00	ND	40	139	1	0.00	0.00	P	+	+	+
Af46	20	108	1	0.00	0.00	ND	19	104–108	2	0.05	0.51	0.906	39	104–108	2	0.03	0.03	P	+	+	+

Note: – = no amplification; + = successful amplification; *A* = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium; M = monomorphic; *N* = sample size; ND = not done; P = polymorphic.

[a] Mexican samples are from Puebla (18.8°N, 99°W), with an extra herbarium specimen from Oaxaca, also in southern Mexico (16.302°N, 96.286°W). Australian samples are from a broader range of populations across northwestern Australia (latitude range 14.463–21.629°S, longitude range 114.918–132.259°E).

[b] Allele size range is the size of the PCR product including the 454A sequencing tag.

* Indicates significance after corrections for multiple tests.

cross-amplification of these loci for a broad range of *Vachellia* species, the primers may be useful for studies of the genetic diversity of other *Vachellia* species.

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Appendix 1. Locality data for the seven individuals of *Vachellia farnesiana* used in the initial screening of 47 loci.

Country	Collection locality	Geographic coordinates
USA	Arizona: Maricopa County	33.2932°N, 112.428°W
Mexico	Veracruz: Los Negritas	18.8383°N, 96.07°W
Mexico	San Luis Potosí	22.2°N, 101°W
Madagascar	Antsiranana: Diana: south of Diego Suarez	12.4321°S, 49.3567°E
Madagascar	Nosy Be	13.3833°S, 48.2°E
Australia	Queensland: 31 km W of Cloncurry	20.7584°S, 140.2327°E
Australia	Western Australia: 180 km E of Halls Creek	17.944°S, 128.8816°E

Appendix 2. Voucher information for *Vachellia* species used in this study.

Species	Voucher specimen accession no.[a]	Collection locality	Geographic coordinates	No. of individuals
Vachellia farnesiana	MO6178804[b]	Oaxaca, Mexico	16.302°N, 96.286°W	1
V. farnesiana	K.L. Bell 128[c]	Katherine, Northern Territory, Australia	14.463°S, 132.259°E	1
V. farnesiana	ASU 279693[d]	Maricopa County, Arizona, USA	33.2932°N, 112.428°W	1
V. farnesiana	MEL 2370354A	Diana, Antsiranana, Madagascar	12.4321°S, 49.3567°E	1
V. aroma	MEL 2263911	Bolivia	20.105°S, 63.487°W	1
V. aroma	MEL 2263912	Bolivia	17.9°S, 64.558°W	1
V. ditricha	MEL 2066644	Wyndham-East Kimberley, Western Australia, Australia	16.3839°S, 126.4975°E	1
V. suberosa	MEL 2066645	Wyndham-East Kimberley, Western Australia, Australia	16.3839°S, 126.4975°E	1
V. nilotica	MEL 260774	Queensland, Australia	21.267°S, 141.3°E	1
V. nilotica	MEL 2080462	Queensland, Australia	20.05°S, 148.25°E	1
V. nilotica	MEL 2204859	Western Australia, Australia	15.803°S, 128.75°E	1
V. nilotica	MEL 2293312	Queensland, Australia	23.446°S, 150.439°E	1

[a] Lodged at the National Herbarium of Victoria (MEL), except where noted.
[b] Lodged at the Missouri Botanical Garden (MO).
[c] Lodged at MEL, but not yet accessioned.
[d] Lodged at the Arizona State University Vascular Plant Herbarium (ASU).